

FORM PTO-1390  
(REV. 1-98)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER

UBC.P-020

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/913325

INTERNATIONAL APPLICATION NO.  
PCT/US00/04875INTERNATIONAL FILING DATE  
25 February 2000PRIORITY DATE CLAIMED  
26 February 1999TITLE OF INVENTION  
TRPM-2 Antisense Therapy

APPLICANT(S) FOR DO/EO/US

Martin Gleave, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau)
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired
  - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3))
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).


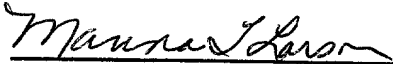
Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
13. ☒ A FIRST preliminary amendment.  
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

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"SECRET" 52 FEB 2001

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U.S. APPLICATION NO. (If known) sec 37 CFR 1.101 <b>09/913325</b>		INTERNATIONAL APPLICATION NO PCT/US00/04875		ATTORNEY'S DOCKET NUMBER UBC.P-020	
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... \$1070.00  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... \$930.00  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$790.00  International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$720.00  International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$98.00  ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	34 - 20 =	14	x \$22.00	\$ 252.00	
Independent claims	4 - 3 =	1	x \$82.00	\$ 80.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				\$ + \$270.00	
TOTAL OF ABOVE CALCULATIONS =				\$ 432.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$ + 216.00	
SUBTOTAL =				\$ 216.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				\$ 216.00	
				Amount to be refunded: \$	
				charged: \$	
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>216.00</u> to cover the above fees is enclosed.					
b. <input checked="" type="checkbox"/> Please charge my Deposit Account No. <u>15-0610</u> in the amount of \$ <u>— 0 —</u> to cover the above fees A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>15-0610</u> . A duplicate copy of this sheet is enclosed					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status					
SEND ALL CORRESPONDENCE TO: OPPEDAHL & LARSON LLP					
 <b>021121</b> PATENT TRADEMARK OFFICE			 SIGNATURE MARINA T. LARSON PH.D. NAME 32,038 REGISTRATION NUMBER		

09/913325  
510 Rec'd PCT/PTO 10 AUG 2001

UBC.P-020  
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Gleave et al.  
Serial No.: to be assigned  
(national phase of PCT/US00/04875)  
Filed: herewith  
For: TRPM-2 Antisense Therapy

PRELIMINARY AMENDMENT

Preliminary to the examination of the above-referenced application, please make the following amendments:

In the specification:

Page 1, please delete the first full paragraph and substitute the following:

-- This application is a Section 371 national phase application based upon International Patent Application No. PCT/US00/04875, filed February 25, 2001, and claims the benefit of US Provisional Application No. 60/121,726, filed February 26, 1999, which is incorporated herein by reference.--

In the claims:

Please amend claims 12 and 14 to read as follows:

12. (amended) The method of claim 8, further comprising the step of administering to the individual a chemotherapy agent.

14. (amended) The method of claim 8, further comprising the step of administering to the individual a second antisense oligodeoxynucleotide which inhibits expression of an anti-apoptotic protein other than TRPM-2.

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Please add claims 29-34 as follows:

29. (new) The method of claim 9, further comprising the step of administering to the individual a chemotherapy agent.

30. (new) The method of claim 9, further comprising the step of administering to the individual a second antisense oligodeoxynucleotide which inhibits expression of an anti-apoptotic protein other than TRPM-2.

31. (new) The method of claim 10, further comprising the step of administering to the individual a chemotherapy agent.

32. (new) The method of claim 10, further comprising the step of administering to the individual a second antisense oligodeoxynucleotide which inhibits expression of an anti-apoptotic protein other than TRPM-2.

33. (new) The method of claim 11, further comprising the step of administering to the individual a chemotherapy agent.

34. (new) The method of claim 11, further comprising the step of administering to the individual a second antisense oligodeoxynucleotide which inhibits expression of an anti-apoptotic protein other than TRPM-2.

REMARKS

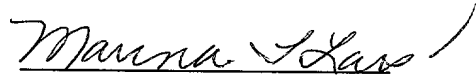
The specification has been amended to provide a reference to the PCT application of which this is a national phase application. In addition, the claims have been amended to eliminate multiple dependency. No new matter has been added, and no new issues are raised. Thus, the Application is entitled to the reduced filing fee and expedited consideration inasmuch

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PATENT APPLICATION

as the IPER prepared in the IPEA/US indicated that all the requirements of PCT Article 33 (1)-(4) were met.

Applicants enclose a copy of the Abstract from the PCT application. In the event that the original abstract was removed from the application for printing, please insert this abstract in the application to conform with US rules requiring a copy of the abstract on a separate sheet.

Respectfully submitted,



Marina T. Larson, Ph.D.

Attorney for Applicant

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MARKED UP COPY OF AMENDED CLAIMS

12. (amended) The method of [any of claims 8-11] claim 8, further comprising the step of administering to the individual a chemotherapy agent.

14. (amended) The method of [any of claims 8-11] claim 8, further comprising the step of administering to the individual a second antisense oligodeoxynucleotide which inhibits expression of an anti-apoptotic protein other than TRPM-2.

TRPM-2 ANTISENSE THERAPY

This application claims priority from US Provisional Patent Application No. 60/121,726, filed February 26, 1999, which is incorporated herein by reference.

Background of the Invention

This application relates to antisense treatments for cancer making use of an antisense oligonucleotide that binds to testosterone-repressed prostate message-2 (TRPM-2).

Prostate cancer is the most common cancer that affects men, and the second leading cause of cancer deaths in men in the Western world. Because prostate cancer is an androgen-sensitive tumor, androgen withdrawal, for example via castration, is utilized in some therapeutic regimens for patients with advanced prostate cancer. Androgen withdrawal leads to extensive apoptosis in the prostate tumor, and hence to a regression of the disease. However, castration-induced apoptosis is not complete, and a progression of surviving tumor cells to androgen-independence ultimately occurs. This progression is the main obstacle to improving survival and quality of life, and efforts have therefore been made to target androgen-independent cells. These efforts have focused on non-hormonal therapies targeted against androgen-independent tumor cells (Yagoda et al., *Cancer* 71 (Supp. 3): 1098-1109 (1993); Oh et al., *J. Urol.* 60: 1220-1229 (1998)), however, so far no non-hormonal agent has improved survival. Alternative approaches are therefore indicated.

It has been observed that numerous proteins are expressed in increased amounts by prostate tumor cells following androgen withdrawal. At least some of these proteins are assumed to be associated with the observed apoptotic cell death which is observed upon androgen withdrawal. (Raffo et al., *Cancer Res.*: 4448-4445 (1995); Krajewska et al., *Am. J. Pathol.* 148: 1567-1576 (1996); McDonnell et al., *Cancer Res.* 52: 6940-6944 (1992)). The functions of many of the proteins, however, is not clearly understood. TRPM-2 (also known as sulfated glycoprotein-2 (SGP-2) or clusterin) is within this latter category.

TRPM-2 is a ubiquitous protein, with a diverse range of proposed activities. In prostate epithelial cell, expression of TRPM-2 increases immediately following castration, reaching peak levels in rat prostate cells at 3 to 4 days post castration, coincident with the onset of massive

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cell death. These results have led some researchers to the conclusion that TRPM-2 is a marker for cell death, and a promoter of apoptosis. On the other hand, the observation that Sertoli cells and some epithelial cells express high levels of TRPM-2 without increased levels of cell death, raises questions as to whether this conclusion is correct.

Sensibar et al., *Cancer Research* 55: 2431-2437 (1995) reported on *in vitro* experiments performed to more clearly elucidate the role of TRPM-2 in prostatic cell death. They utilized LNCaP cells transfected with a gene encoding TRPM-2 and observed whether expression of this protein altered the effects of tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), to which LNCaP cells are very sensitive, with cell death normally occurring within about 12 hours. Treatment of the transfected LNCaP cells with TNF $\alpha$  was shown to result in a transient increase in TRPM-2 levels for a period of a few hours, but these levels had dissipated by the time DNA fragmentation preceeding cell death was observed. Using an antisense molecule corresponding to the bases 1-21 of the TRPM-2 sequence, but not other TRPM-2 antisense oligonucleotides, resulted in a substantial reduction in expression of TRPM-2, and an increase in apoptotic cell death in LNCaP cells exposed to TNF $\alpha$ . This led Sensibar et al. to the hypothesis that overexpression of TRPM-2 could protect cells from the cytotoxic effect of TNF, and that TRPM-2 depletion is responsible for the onset of cell death, although the mechanism of action remains unclear.

While Sensibar et al. provides information about the possible role of TRPM-2, it nevertheless discloses results from only a model system in which expression of TRPM-2 is based on a transfected gene. Furthermore, expression levels of TRPM-2 is very low or absent in LNCaP cells grown in other labs. The situation which results *in vivo* when prostate tumor cells are subjected to androgen withdrawal is far more complex, with numerous proteins changing expression levels as a result. Thus, it is not possible from the Sensibar et al. data to predict whether TRPM-2 would perform the same function when present in combination with other proteins, or whether changes in levels of TRPM-2 following androgen withdrawal *in vivo* could provide any therapeutic benefits. Indeed, the fact that TRPM-2 is expressed in substantial quantities in prostatic tumor cells at various stages following androgen withdrawal, including stages where significant apoptotic cell death is occurring suggests that role of TRPM-2 *in vivo* may be more complicated. Thus, while the art provides data concerning certain aspects of apoptotic cell death in prostatic tumor cells, it offers

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neither a teaching or a suggestion of a methodology to provide a delay in the onset of androgen-independence.

It is an object of the present invention to provide such a method.

It is a further object of the present invention to provide therapeutic antisense molecules for delaying the onset of androgen independence in prostatic tumor cells.

It is an additional object of the present invention to provide a method for enhancing the chemosensitivity or radiation sensitivity of cancer cells from a cancer that expresses TRPM-2.

It is a further object of the present invention to provide therapeutic antisense molecules for inhibiting expression of TRPM-2.

#### Summary of the Invention

In accordance with the present invention, it has now been determined that antisense therapy which reduces the expression of TRPM-2 provides therapeutic benefits in the treatment of cancer. In particular, such antisense therapy can be applied in treatment of prostate cancer and renal cell cancer.

Addition of antisense TRPM-2 oligodeoxynucleotide (ODN) to prostatic tumor cells *in vivo* is effective for delaying the onset of androgen independence. Thus, in one aspect, the invention provides a method for treating prostate cancer in an individual suffering from prostate cancer, comprising the steps of initiating androgen-withdrawal to induce apoptotic cell death of prostatic tumor cells in the individual, and administering to the individual a composition effective to inhibit expression of TRPM-2 by the tumor cells, thereby delaying the progression of prostatic tumor cells to an androgen-independent state in an individual. Furthermore, combined use of antisense TRPM-2 plus cytotoxic chemotherapy (e.g. taxanes) synergistically enhances chemosensitivity in hormone refractory prostate cancer. In another aspect of the invention, a second antisense ODN which inhibits expression of an anti-apoptotic protein other than TRPM-2 is administered along with the antisense TRPM-2 ODN.

It has also been found that antisense TRPM-2 has beneficial effects for other cancer types. Specifically, antisense TRPM-2 ODN enhances chemosensitivity in human Renal cell cancer, a normally chemoresistant disease with no active chemotherapeutic agent having an

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objective response rate higher than 10%. Radiation sensitivity is also enhanced when cells expressing TRPM-2 are treated with antisense TRPM-2 ODN. Thus, the antisense TRPM-2 ODNs can be used to treat a variety of cancer types in which expression of TRPM-2 has been observed.

#### Brief Description of the Figures

Fig. 1 shows the delay in onset of androgen-independence which is achieved using an antisense TRPM-2 ODN;

Fig. 2 shows the positions of 10 antisense oligonucleotides evaluated for the ability to inhibit TRPM-2 expression and delay onset of androgen-independence;

Fig. 3 shows expression levels of TRPM-2 mRNA in the presence of various antisense ODNs;

Fig. 4 shows the levels of TRPM-2 mRNA in Shionogi cells treated *in vitro* with varying amounts of antisense TRPM-2 ODN or a mismatch control;

Fig. 5 shows the dose-response curve for combinations of taxol and antisense TRPM-2 ODN;

Fig. 6 shows the dose-response curve for combinations of taxol, antisense TRPM-2 ODN and antisense Bcl-2 ODN;

Fig. 7A shows decrease in TRPM-2 mRNA levels in human renal cell cancer after treatment with antisense TRPM-2 ODNs;

Fig. 7B shows the increase in chemosensitivity of human renal cell cancer to taxol after treatment with antisense TRPM-2 ODNs;

Fig. 8 shows TRPM-2 expression in PC-3 prostate cancer cells after various doses of radiation;

Figs. 9A and 9B show the comparative radiation resistance of human prostate cell lines which overexpress (LNCaP/T) and normally (LNCaP/P) express TRPM-2;

Fig. 10 shows the increased susceptibility of PC-3 cells to radiation after treatment with antisense TRPM-2 ODN; and

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Figs. 11A and 11B show the increased sensitivity of PC-3 cells to radiation after treatment with antisense TRPM-2 ODN.

Figs. 12A and 12B show the increased sensitivity of Shionogi tumor cells to chemotherapy agents paclitaxel and mitoxanthrone when administered with antisense TRPM-2 ODN.

### Detailed Description of the Invention

The present invention relates to antisense TRPM-2 ODNs and to the use of these compositions in the treatment of cancer. The invention can be applied in the treatment of cancers where the cancer cells express TRPM-2. Three significant classes of cancer cells which express TRPM-2 are prostate cancer cells, human renal cell cancer (RCC) cells and some breast cancer cells.

In one embodiment, the present invention provides a method for enhancing castration-induced tumor cell death and delaying the progression of prostatic tumor cells to androgen independence; a therapeutic method for the treatment of individuals, including humans, suffering from prostate cancer; and therapeutic agents effective for use in such methods. The therapeutic method of the invention will most commonly be used in the treatment of individuals with advanced prostate cancer.

Enhancement of castration-induced tumor cell death and delay of the progression of androgen-sensitive prostatic cancer cells to androgen-independent is achieved by inhibiting the expression of TRPM-2 by the cells. Experiments were performed in three model systems, the *in vivo* Shionogi tumor model, the human TRPM-2 transfected LNCaP model, and the human PC-3 model, which taken together demonstrated that such inhibition leading to delay of androgen-independence can be achieved by treating androgen-sensitive prostatic tumor cells with antisense oligodeoxynucleotides (ODNs).

In the first experiment, the ability of a mouse TRPM-2 antisense molecule, (Seq. ID. No. 1) to delay onset of androgen independence in the Shionogi tumor model was evaluated. The Shionogi tumor model is a xenograft of an androgen-dependent mouse mammary carcinoma that grows subcutaneously in male syngeneic hosts. Shionogi tumor cells are highly tumorigenic and

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locally invasive. The cells have been shown to respond to androgen withdrawal in a manner which mimics the observed behavior of prostatic tumor cells, and have been accepted as a valid model for prostate cancer in humans. (Bruchovsky et al., *Cancer Res.* 50: 2275-2282 (1990); Rennie et al., *Cancer Res.* 48: 6309-6312 (1988); Bruchovsky et al., *Cell* 13: 272-280 (1978); Gleave et al., in *Genitourinary Oncology*, pp. 367-378, Lange et al., eds, Lippencott (1997); Gleave et al., *J. Urol.* 157: 1727-1730 (1997); Bruchovsky et al., *The Prostate* 6: 13-21 (1996)). Thus, androgen withdrawal precipitates apoptosis and tumor regression in a highly reproducible manner. Further, changes in expression of TRPM-2 and Bcl-2 in human prostate cancer following castration and during progression to androgen independence are similar to those observed in Shionogi tumor cells. Thus, the Shionogi tumor model mimics many of the characteristics of prostate cancer cells. Further, the Shionogi tumor model provides a very useful model for the evaluation of the ability of compounds to delay the onset of androgen-independence. Despite complete tumor regression after castration, rapidly growing androgen-independent Shionogi tumors invariably recur after one month, which provides a reliable end point to evaluate agents which can delay the progression to androgen-independence. In general, events which occur in the Shionogi tumor model within one month occur in human patients within about two years.

The ability of the antisense ODNs that inhibit expression of TRPM-2 to delay the onset of androgen-independence was evaluated by measuring tumor volume post-castration in the Shionogi tumor model. The test animals (n=7) were treated intraperitoneally once daily with 12.5 mg/kg repeat doses of antisense TRPM-2 ODNs (Seq. ID. No. 1) in a buffered saline solution. As a control, animals (n=7) were treated with a mismatch ODN (Seq. ID. No. 2). As shown in Fig. 1, both test and control groups showed the expected decline in tumor volume immediately following castration, but the tumors in the antisense TRPM-2 ODN-treated mice regressed faster than the controls. The control group also exhibited the expected increase in tumor volume which is associated the development of androgen-independence. In contrast, at 49 days post-castration, little tumor regrowth had occurred in the mice treated using the antisense TRPM-2 ODN. Tumors did eventually recur in the antisense TRPM-2 ODN-treated mice, but the median time to recurrence is approximately twice that of the control group. Thus, inhibition of TRPM-2 is effective not only for increasing the amount of cell death which occurs immediately following androgen withdrawal,

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but also for delaying the onset of androgen-independence. The more rapid decrease in tumor volume in the mice treated with antisense TRPM-2 ODNs was due to earlier onset and more extensive castration-induced apoptosis. This was confirmed by detecting poly(ADP-ribose) polymerase (PARP) cleavage fragments in Shionogi tumor specimens (Miyake, et al., *Cancer Res.* 60:170-176 (2000)).

To evaluate which human antisense ODNs complementary to TRPM-2 mRNA sequences are most effective for this purpose, a series of ten antisense phosphorothioate ODNs were prepared spanning various mRNA regions as shown in Fig. 2. The sequences of these ten ODNs are set forth in the attached Sequence Listing as Seq. ID. Nos. 3-12. The ten human antisense ODNs were evaluated using TRPM-2 transfected LNCaP cells and human prostate cancer PC-3 cells for their ability to inhibit expression of TRPM-2 mRNA. As shown in Fig. 3, the antisense ODNs tested produced variable levels of inhibition of TRPM-2 mRNA expression, with the best results being achieved with Seq. ID Nos. 4, 5, and 12. Sequence ID No. 5 corresponds to the sequence used by Sensibar et al. that produced inhibition of TRPM-2 expression in LNCaP cells, and is complementary to the first 21 bases of the TRPM-2 mRNA. The most effective down-regulation occurred with Seq. ID No. 4. Common to all of the effective sequences is an overlap with either the initiation or termination sites of the TRPM-2 mRNA. Thus, in a general sense, the method of the invention can be practiced with antisense oligonucleotides which are complementary to a region of the TRPM-2 mRNA spanning either the translation initiation site or the termination site.

In accordance with a further aspect of with the invention, therapeutic treatment of individuals, including human individuals, suffering from prostate cancer is achieved by initiating androgen-withdrawal to induce apoptotic cell death of prostatic tumor cells in the individual, and administering to the individual a composition effective to inhibit expression of TRPM-2 by the tumor cells, thereby delaying the progression of prostatic tumor cells to an androgen-independent state in an individual.

Initiation of androgen withdrawal may be accomplished via surgical (removal of both testicles) or medical (drug-induced suppression of testosterone) castration, which is currently indicated for treatment of prostate cancer. Medical castration can be achieved by various regimens,

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including LHRH agents or antiandrogens. (Gleave et al., *CMAJ* 160: 225-232 (1999)).

Intermittent therapy in which reversible androgen withdrawal is effected is described in Gleave et al. *Eur. Urol.* 34 (Supp. 3): 37-41 (1998).

The inhibition of TRPM-2 expression may be transient, and ideally should occur coincident with androgen withdrawal. In humans, this means that inhibition of expression should be effective starting within a day or two of androgen withdrawal and extending for about 3 to 6 months. This may require multiple doses to accomplish. It will be appreciated, however, that the period of time may be more prolonged, starting before castration and extending for substantial time afterwards without departing from the scope of the invention.

Antisense TRPM-2 ODNs have also been determined to enhance chemosensitivity in human renal cell cancer (RCC). RCC is a chemoresistant disease with no active chemotherapeutic agent with objective response rates higher than 10 %. Increased TRPM-2 expression in renal proximal convoluted cells undergoing apoptosis has been observed after various stimuli including ureteral obstruction and aminoglycosides. However, functional significance of TRPM-2 expression in RCC has not been well documented. Test results show, however, that antisense TRPM-2 ODN enhances chemosensitivity in human RCC CaKi-2 cells (See Example 6, *infra*).

Antisense TRPM-2 ODNs were also found to increase sensitivity to radiation (See Example 7 and Fig. 8).

Inhibition of expression of TRPM-2 may be accomplished by the administration of antisense ODNs, particularly antisense ODNs which are complementary to a region of the TRPM-2 mRNA spanning either the translation initiation site or the termination site. For treatment of prostate cancer in humans, specific useful sequences are those shown in Seq. ID Nos. 4, 5 and 12.

The ODNs employed may be modified to increase the stability of the ODN *in vivo*. For example, the ODNs may be employed as phosphorothioate derivatives (replacement of a non-bridging phosphoryl oxygen atoms with a sulfur atom) which have increased resistance to nuclease digestion. MOE modification (ISIS backbone) is also effective.

Administration of antisense ODNs can be carried out using the various mechanisms known in the art, including naked administration and administration in pharmaceutically acceptable

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lipid carriers. For example, lipid carriers for antisense delivery are disclosed in US Patents No. 5,855,911 and 5,417,978 which are incorporated herein by reference. In general, the antisense is administered by intravenous, intraperitoneal, subcutaneous or oral routes, or direct local tumor injection. From the experiments performed using the Shionogi mouse model, it appears that the antisense ODN is preferentially active in the tumor cells. Indeed, TRPM-2 expression in non-tumor tissues was substantially unaffected, and no side effects of the antisense ODN administration were observed.

The amount of antisense ODN administered is one effective to inhibit the expression of TRPM-2 in prostatic cells. It will be appreciated that this amount will vary both with the effectiveness of the antisense ODN employed, and with the nature of any carrier used. The determination of appropriate amounts for any given composition is within the skill in the art, through standard series of tests designed to assess appropriate therapeutic levels. .

The method for treating prostate cancer in accordance with the invention may further include administration of chemotherapy agents and/or additional antisense ODNs directed at different targets. For example, it has been found using the Shionogi tumor model that antisense TRPM-2 ODN increases sensitivity to conventional chemotherapy agents such as taxanes (paclitaxel or docetaxel) and mitoxanthrone (Figs. 12A and 12B). As shown in Figs. 12A and 12B, treatment with antisense TRPM-2 ODN in the presence of taxol or mitoxanthrone resulted in a reduced tumor volume as compared to the combination of taxol or mitoxanthrone with the mismatch (MM) ODN. Other agents likely to show synergistic activity include other cytotoxic agents (e.g. cyclophosphamide, topoisomerase inhibitors), angiogenesis inhibitors, differentiation agents and signal transduction inhibitors. Similarly, combinations of TRPM-2 antisense with other antisense species such as antisense Bcl-2 ODN worked better at killing Shionogi cells *in vitro* than either ODN alone. Thus, TRPM-2 can work in concert with other antisense molecules, such as antisense Bcl-2, Bcl-xl and c-myc ODN to provide greater effectiveness.

The invention will now be further described with reference to the following, non-limiting examples.

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EXAMPLE 1

Shionogi tumor model experiments were performed using cells from the Toronto subline of transplantable SC-115 AD mouse mammary carcinoma. For *in vivo* studies, approximately  $5 \times 10^6$  cells of the Shionogi carcinoma were injected subcutaneously in adult male DD/S strain mice. When the Shionogi tumors became 1 to 2 cm in diameter, usually 2 to 3 week after injection, castration was performed through an abdominal incision under methoxyflurane anesthesia. Details of the maintenance of mice, tumor stock and operative procedures have been previously described. Bruchovsky et al., *Cancer res.* 50: 2275-2282 (1990); Rennie et al., *Cancer Res.* 48: 6309-6312 (1988); Bruchovsky et al., *Cell* 13: 272-280 (1978); Gleave et al., in *Genitourinary Oncology*, pp. 367-378, Lange et al., eds, Lippencott (1997); Gleave et al., *J. Urol.* 157: 1727-1730 (1997); Bruchovsky et al., *The Prostate* 6: 13-21 (1996)).

Mice were randomly selected for treatment with murine phosphorothioate antisense TRPM-2 ODN (Seq. ID No. 1) or a mismatch control (Seq. ID No. 2) which is two bases different in sequence from the antisense TRPM-2 ODN. Each experimental group consisted of 7 mice. One day after castration, 12.5 mg/kg of antisense TRPM-2 or mismatch control ODN dissolved in phosphate buffered saline were injected intraperitoneally once daily into each mouse of 40 days. Tumor volume was measured twice weekly, and calculated by the formula length X width X depth X 0.5236. Gleave et al., *Cancer Res.* 52: 1598-1605 (1992). Data points were reported as average tumor volumes  $\pm$  standard deviation.

The results of this study are shown in Fig. 1. As shown, Shionogi tumors regressed faster and complete regression occurred earlier in mice treated with antisense TRPM-2 ODN. Furthermore, treatment with antisense TRPM-2 ODN substantially delayed the onset of androgen-independence which is reflected by the increase in tumor volume after day 21 in the control animals. No side effects associated with antisense TRPM-2 or the mismatch control were observed.

To examine the effects of *in vivo* ODN treatment on levels of TRPM-2 mRNA, Northern blot analysis was performed on Shionogi tumor tissue from mice. The mice were treated daily with 12.5 mg/kg of antisense TRPM-2 ODN (n=6) or the mismatch control (n=6) by intraperitoneal injection starting one day after castration. On the fourth day after castration, tumor tissues were harvested and analyzed by Northern blot for TRPM-2 mRNA. Antisense TRPM-2

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ODN resulted in a 75% reduction in TRPM-2 mRNA levels in Shionogi tumors compared to mismatch control ODN treated tumors. (Fig. 3).

Comparable analyses were performed on normal mouse organs. Samples of spleen, kidney, prostate and brain were harvested from Shionogi tumor mice treated with antisense TRPM-2 ODN and mismatch control under the same treatment schedule, and analyzed by Northern blot. Although TRPM-2 mRNA levels was significantly lower in tumor tissues, antisense TRPM-2 ODN had no effect on TRPM-2 mRNA levels in the normal organs.

### EXAMPLE 2

The sequence selectivity of the antisense TRPM-2 ODN (Seq. ID. No. 1) was confirmed by comparing expression levels of TRPM-2 mRNA in Shionogi tumor cells maintained *in vitro*, after treatment with the varying levels of antisense TRPM-2 ODN or a mismatch control (Seq. ID. No. 2). To facilitate uptake of the ODNs into the cells, the ODNs were formulated in a cationic lipid carrier (Lipofectin™, (Life Technologies, Inc.)). Cells were treated twice over a period of two days using the following protocol. Cells were preincubated for 20 minutes with 4 µg/ml of lipofectin in serum free OPTI-MEM™ (Life Technologies, Inc.) and then incubated with the medium containing the selected concentration of ODN and lipofectin for four hours. The medium was then replaced with the standard culture medium.

The amount of TRPM-2 mRNA in the cells was evaluated using Northern blot analysis. As shown in Fig. 4, treatment of Shionogi cells with antisense TRPM-2 ODN reduced TRPM-2 mRNA levels in a dose dependent manner. In contrast, TRPM-2 mRNA levels were not affected by the mismatch ODN (Seq. ID. No. 2) at any of the employed concentrations. Thus, the affect of antisense TRPM-2 ODN is apparently sequence specific.

### EXAMPLE 3

Shionogi cells maintained *in vitro* were treated with varying amounts of taxol alone or in combination with 500 nM antisense TRPM-2 ODN (Seq. ID. No. 1) or the mismatch control (Seq. ID No. 2). The cells were treated twice, as described in Example 2, and the percentage of viable cells remaining was determined. The results are summarized in Fig. 5. As shown, the

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inclusion of antisense TRPM-2 ODN shifted the dose-response curve to the left, lowering the  $IC_{50}$  by a factor of 5 to 10. Similar results were achieved using mitoxanthrone in place of paclitaxel (Figs. 12A and 12B).

#### EXAMPLE 4

The experiment of Example 3 was repeated, with the addition of antisense Bcl-2 ODN (Seq. ID. No. 13) or a mismatch Bcl-2 ODN (Seq. ID. No. 14) in various combinations with antisense/mismatch TRPM-2 ODN and taxol. The results are shown in Fig. 6. The combination of antisense TRPM-2 ODN with antisense Bcl-2 ODN and taxol further enhanced the cytotoxic effects of taxol. Thus, the targeting of additional anti-apoptotic agents appears to provide therapeutic benefits.

#### EXAMPLE 5

To identify appropriate antisense TRPM-2 ODN sequences for use in human therapy, antisense ODN sequences directed against 10 different sites of the human TRPM-2 gene (Fig. 2, Seq. ID Nos. 3-12) were synthesized and tested for their ability to decrease TRPM-2 gene expression in human prostate cancer PC-3 and transfected LNCaP cells that overexpress TRPM-2 using the same treatment protocol described in Example 2. The results are summarized in Fig. 3. As shown, sequences 4, 5 and 12 are active for reduction of TRPM-2 expression. These three sequences overlap or are immediately adjacent to the translation initiation or termination sites.

#### EXAMPLE 6

Immunohistochemical staining was used to characterize clusterin expression in 17 RCC and normal kidney tissues obtained from radical nephrectomy specimens. TRPM-2 expression in human renal cancer cell lines ACHN, CaKi-1 and CaKi-2 was evaluated by Northern and Western blot analyses. Northern blot analysis was used to assess changes in TRPM-2 mRNA expression after antisense TRPM-2 ODN treatment. The effects of combined antisense TRPM-2 ODN and taxol treatment on CaKi-2 cell growth was examined using a MTT assay.

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Immunostaining showed an increased clusterin expression in 11 RCC specimens in comparison to the adjacent normal kidney tissue. In the remaining 6 cases, no difference was seen between malignant and normal tissue. Both TRPM-2 mRNA and protein expression were detectable in all three human RCC cell lines, with highest levels for CaKi-2.

Antisense TRPM-2 ODN (Seq. ID. No. 1), but not mismatch control ODN (Seq. ID. No. 2), inhibited TRPM-2 expression in CaKi-2 cells in a dose dependant and sequence specific manner (Fig. 7A). Furthermore, antisense TRPM-2 ODN substantially enhanced taxol chemosensitivity, reducing IC<sub>50</sub> of taxol by 1 log (500 nM to 50 nM) compared to mismatch control ODN (Fig. 7B). These data demonstrate that TRPM-2 and its protein, clusterin, are expressed at higher levels in RCC compared to normal kidney tissue, and that antisense TRPM-2 ODN may be useful in enhancing the cytotoxic effects of conventional chemotherapy in advanced RCC.

#### EXAMPLE 7

Antisense TRPM-2 ODNs enhance radiation sensitivity of cancer cells which express TRPM-2. Using northern analysis, we found that radiation therapy results in dose and time dependent increases in TRPM2 gene expression in human prostate cancer PC-3 cells (Fig 8). Overexpression of TRPM2 results in increased resistance to radiation induced cell death. Human prostate LNCaP cells that overexpress TRPM2 (LNCaP/T1) are more resistant to radiation therapy (Figs. 9A and B). Treatment of human prostate cancer PC-3 cells with 100 and 500 nM antisense TRPM-2 ODNs (Seq. ID. NO. 1) significantly reduces cell survival after a single treatment of 4 Gy radiation therapy compared to mismatch ODN (Seq. ID No. 2) treatment. (Fig. 10). Figure 11A and B show dose dependent radiation sensitization of human prostate cancer PC-3 cells after treatment with 10, 50, and 100 nM antisense TRPM-2 oligo *in vitro*.

#### EXAMPLE 8

To determine whether treatment with human antisense TRPM-2 ODN enhances chemosensitivity in the PC3 human prostate cancer cell line, mice bearing PC3 tumors were treated with antisense human TRPM-2 ODN plus micellar paclitaxel or mitoxantrone, and mismatch control ODN plus micellar paclitaxel or mitoxantrone (Figs. 12A and 12B). ODN was administered for 28

days and either 0.5 mg micellar taxol or 0.3 mg mitoxantrone were administered on two occasions: from day 10 to 14, and day 24 to 28. A significant reduction in tumor size was observed in the antisense ODN treated animals as compared to those treated with mismatch control ODN. This effect was even more pronounced after the second dosing of the micellar paclitaxel or mitoxantrone.

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-16-

CLAIMS

1. A method for delaying progression of prostatic tumor cells to an androgen-independent state, comprising treating androgen-sensitive prostatic tumor cells *in vivo* with an antisense oligonucleotide which inhibits expression of TRPM-2 by the tumor cells.
2. The method of claim 1, wherein the antisense oligonucleotide is complementary to a region of TRPM-2 mRNA including the translation initiation or termination site.
3. The method of claim 2, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 4.
4. The method of claim 2, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 5.
5. The method of claim 2, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 12.
6. A method for treating prostate cancer in an individual suffering from prostate cancer, comprising the steps of initiating androgen-withdrawal to induce apoptotic cell death of prostatic tumor cells in the individual, and administering to the individual a composition effective to inhibit expression of TRPM-2 by the tumor cells, thereby delaying the progression of prostatic tumor cells to an androgen-independent state in an individual.
7. The method of claim 6, wherein the composition effective to inhibit expression of TRPM-2 is an antisense oligonucleotide.

AMENDED SHEET

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-17-

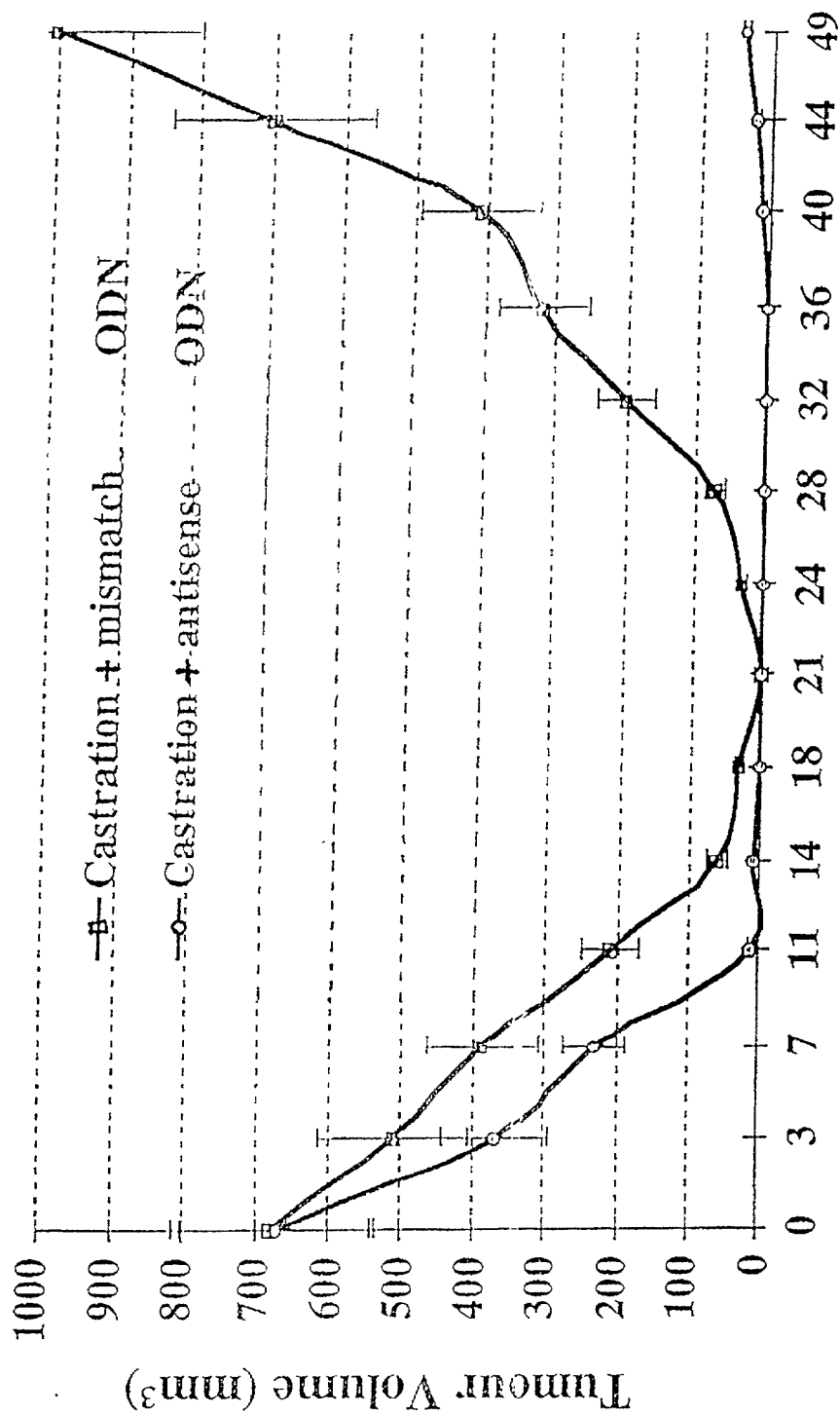
8. The method of claim 7, wherein the antisense oligonucleotide is complementary to a region of TRPM-2 mRNA including the translation initiation or termination site.
9. The method of claim 8, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 4.
10. The method of claim 8, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 5.
11. The method of claim 8, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 12.
12. The method of any of claims 8-11, further comprising the step of administering to the individual a chemotherapy agent.
13. The method of claims 12, wherein the chemotherapy agent is a taxane or mitoxanthrone.
14. The method of any of claims 8-11, further comprising the step of administering to the individual a second antisense oligodeoxynucleotide which inhibits expression of an anti-apoptotic protein other than TRPM-2.
15. The method of claim 14, wherein the second antisense oligodeoxynucleotide is antisense Bcl-2 oligodeoxynucleotide.
16. The method of claim 14, further comprising the step of administering to the individual a chemotherapy agent.
17. The method of claims 16, wherein the chemotherapy agent is a taxane or mitoxanthrone.

00130 08100 00130 08100

17  
-18-

18. A method for enhancing the chemo- or radiation sensitivity of cancer cells in an individual suffering from a cancer that expresses TRPM-2 in amounts different from normal tissue of the same type, comprising administering to the individual a composition effective to inhibit expression of TRPM-2 by cancer cells.
19. The method of claim 12, wherein the composition effective to inhibit expression of TRPM-2 is an antisense oligonucleotide.
20. The method of claim 13, wherein the antisense oligonucleotide is complementary to a region of TRPM-2 mRNA including the translation initiation or termination site.
21. The method of claim 14, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 4.
22. The method of claim 14, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 5.
23. The method of claim 14, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 12.
24. A method of delaying of progression of a population of prostatic tumor cells from a state in which living prostatic tumor cells are androgen-sensitive to a state in which living tumor cells are androgen independent, comprising treating the population of androgen-sensitive prostatic tumor cells with an antisense oligonucleotide which inhibits expression of TRPM-2 by the tumor cells.
25. The method of claim 24, wherein the antisense oligonucleotide is complementary to a region of TRPM-2 mRNA including the translation initiation or termination site.

26. The method of claim 25, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 4.
27. The method of claim 25, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 5.
28. The method of claim 25, wherein the antisense oligonucleotide has the sequence given by SEQ ID No. 12.



Days Post-Castration

Fig 1

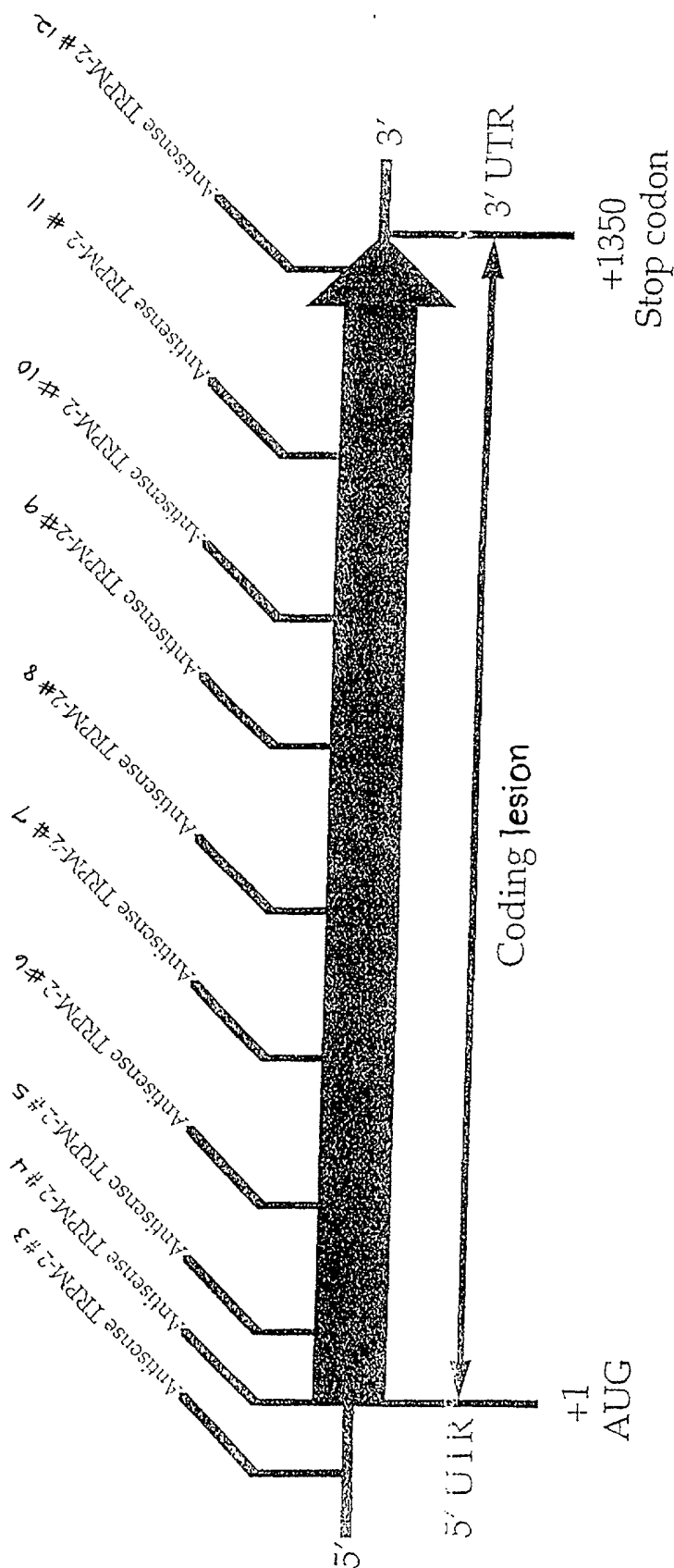


Fig 2

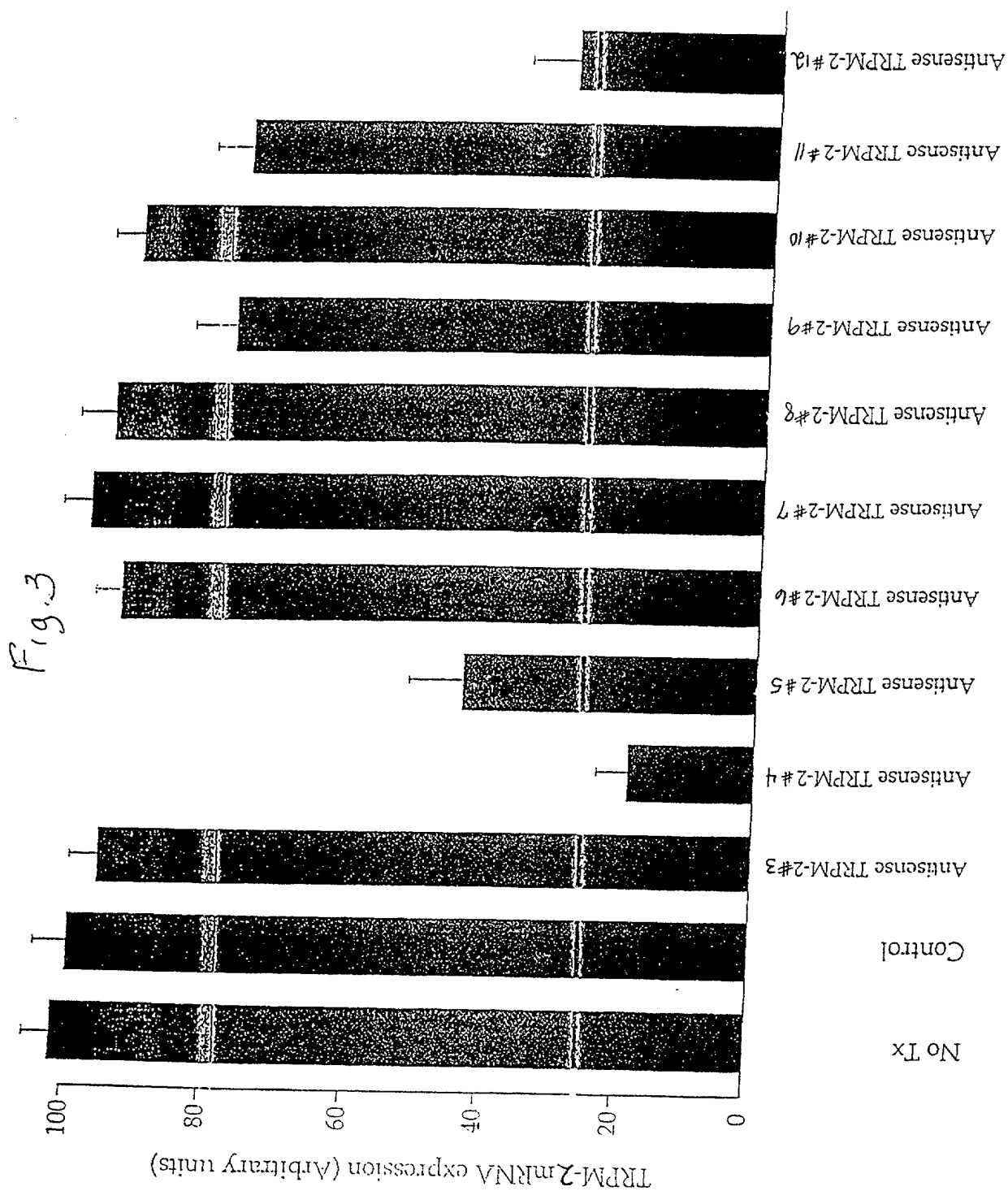


FIG. 3

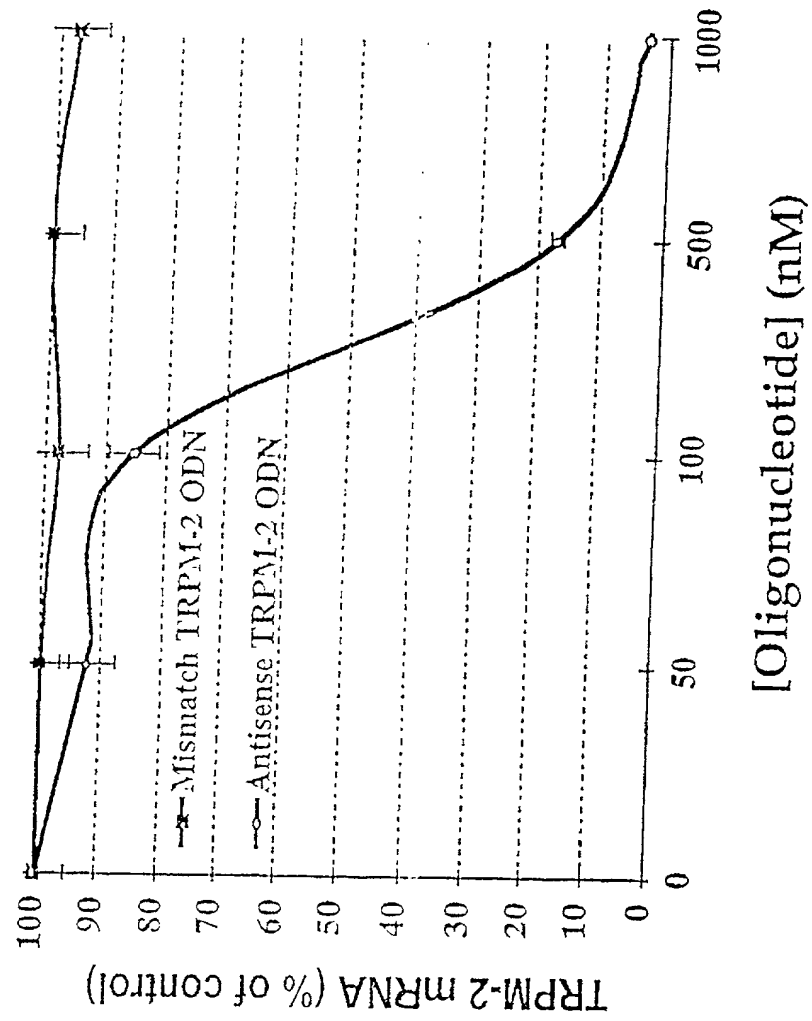


Fig. 4.

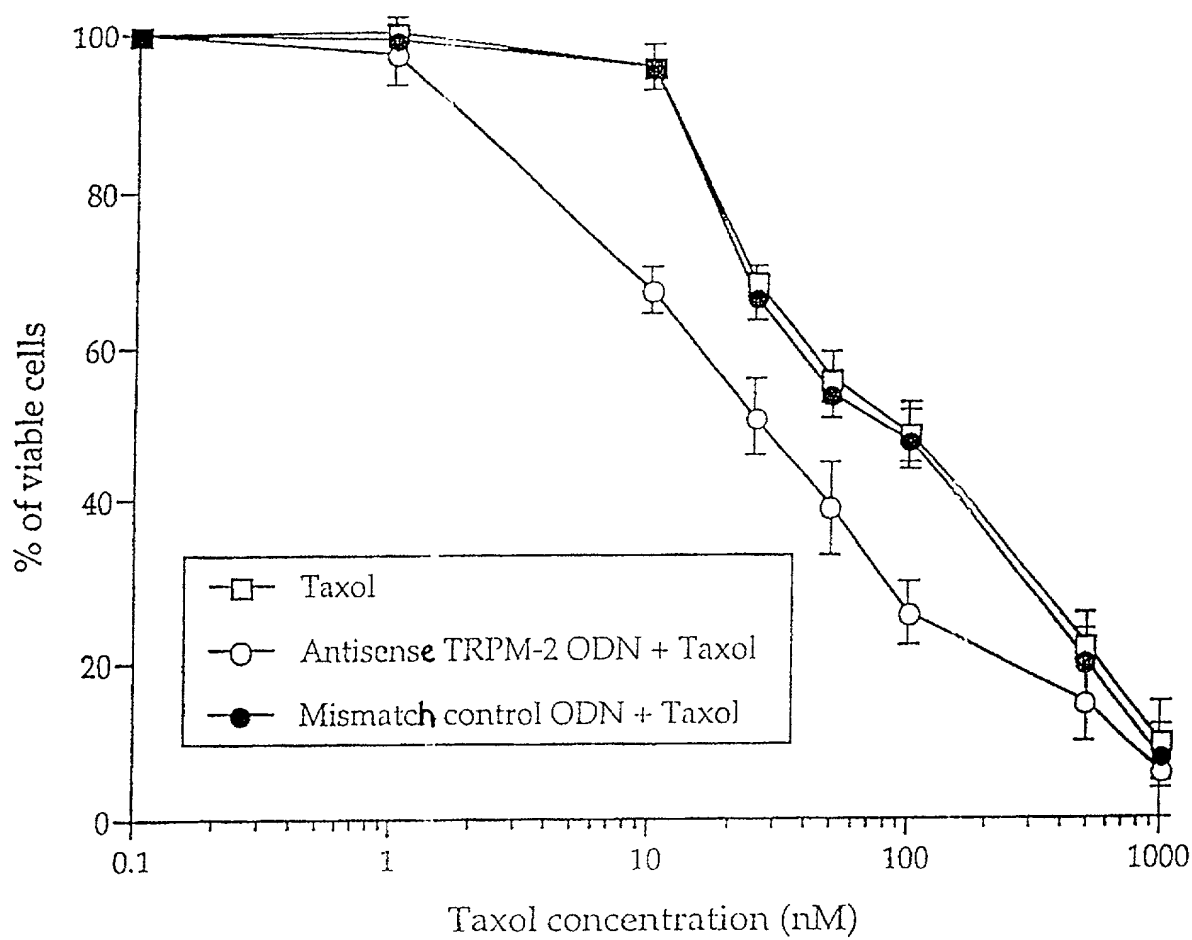


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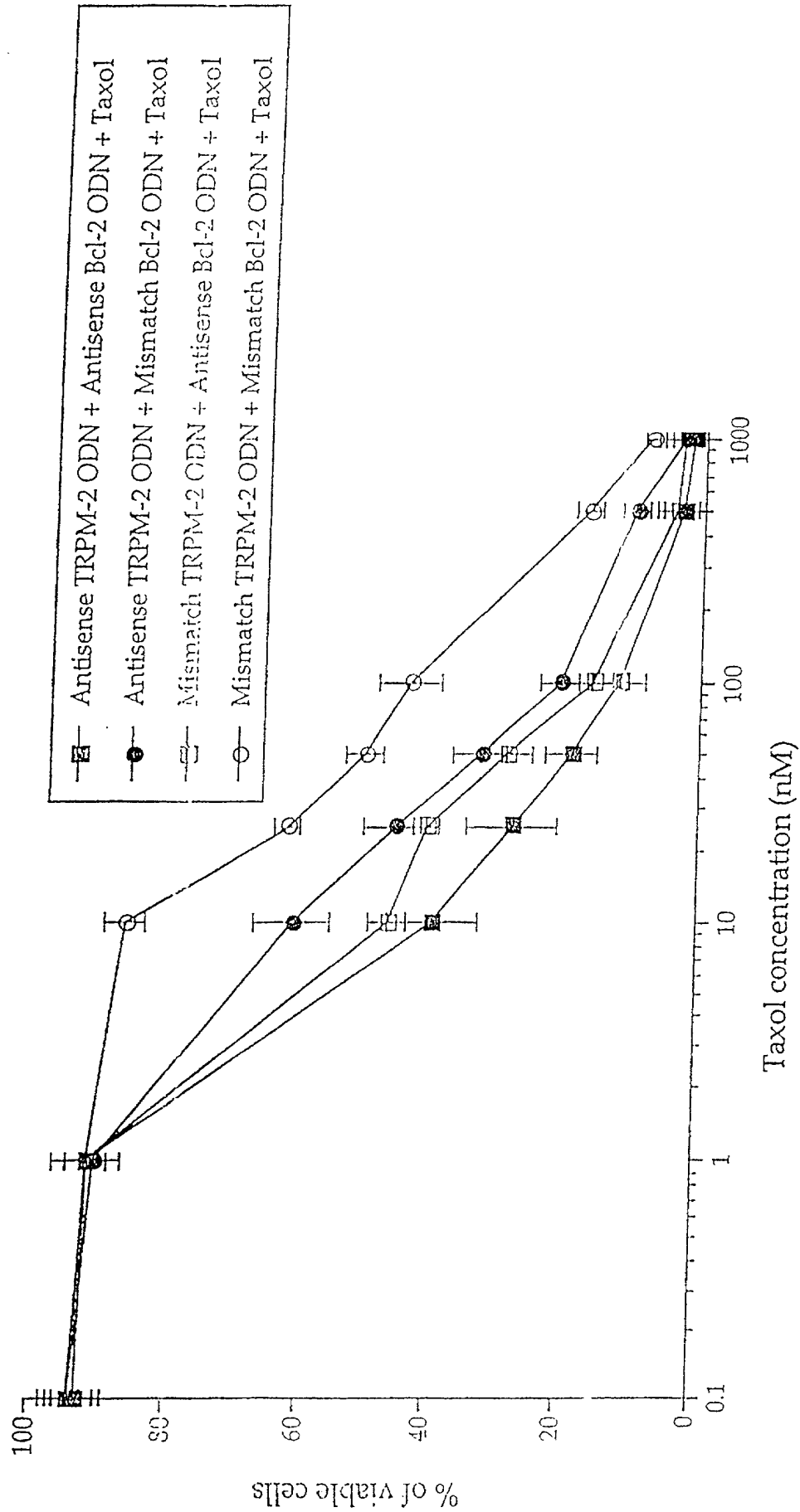


Fig. 6

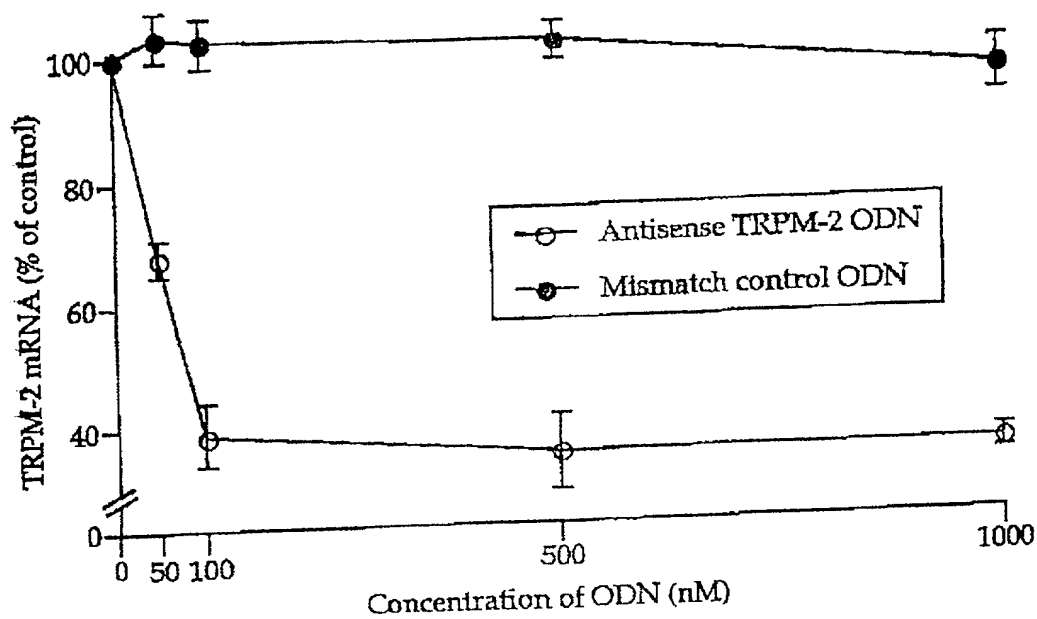


Fig 7A

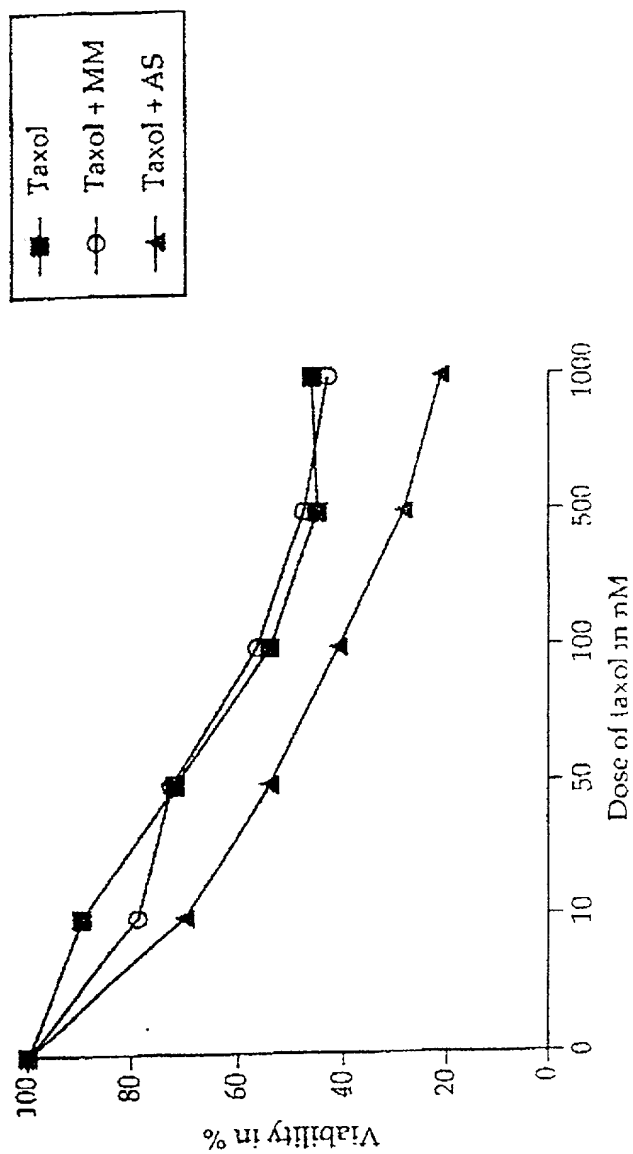


Fig 7B

FIGURE 7B

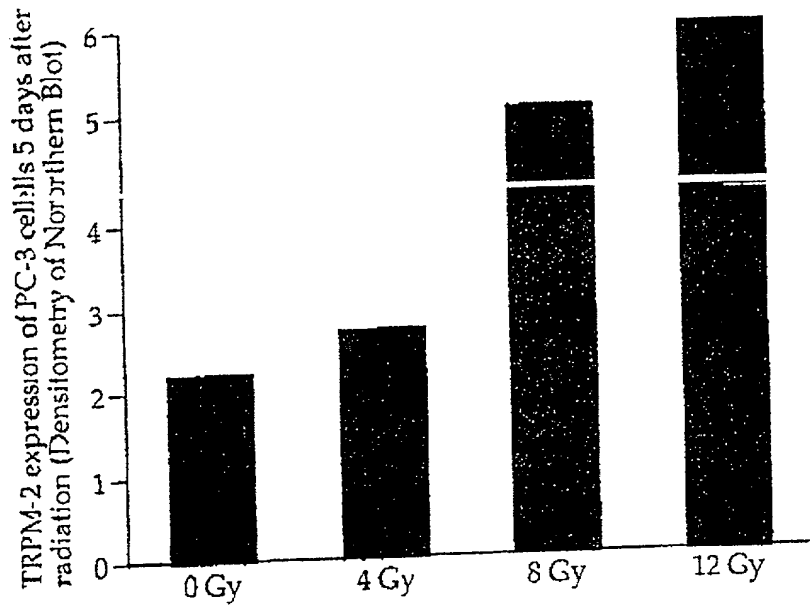


Fig 8 -

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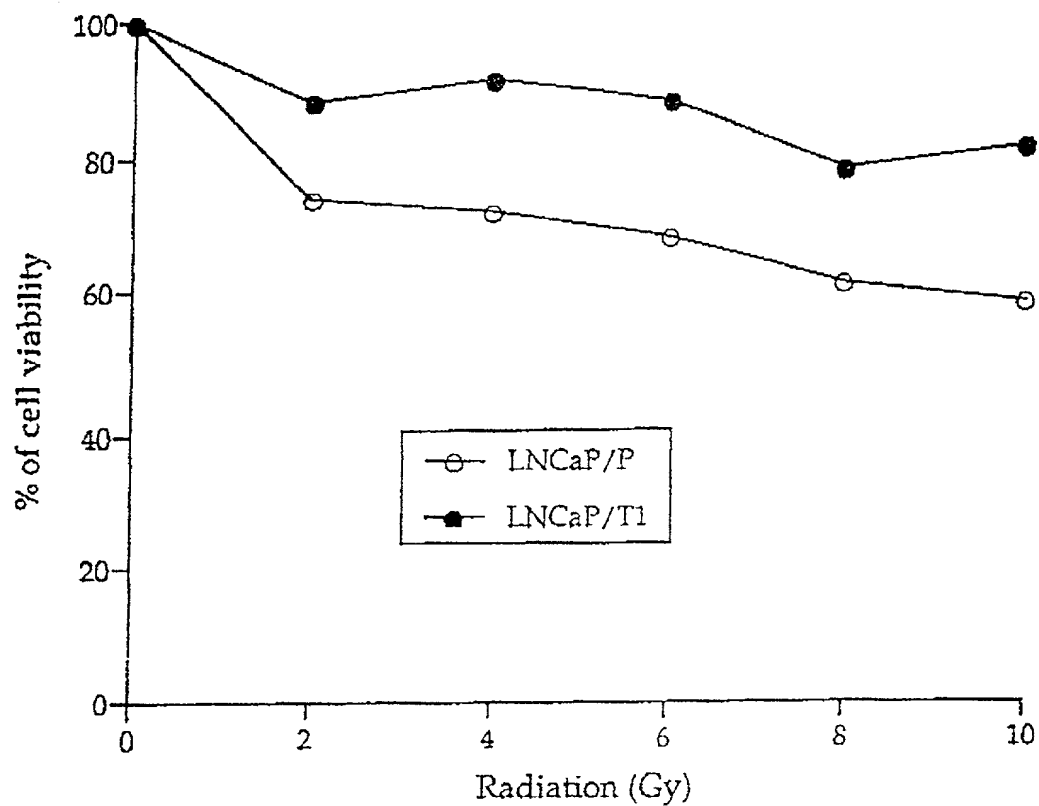


Fig 9A

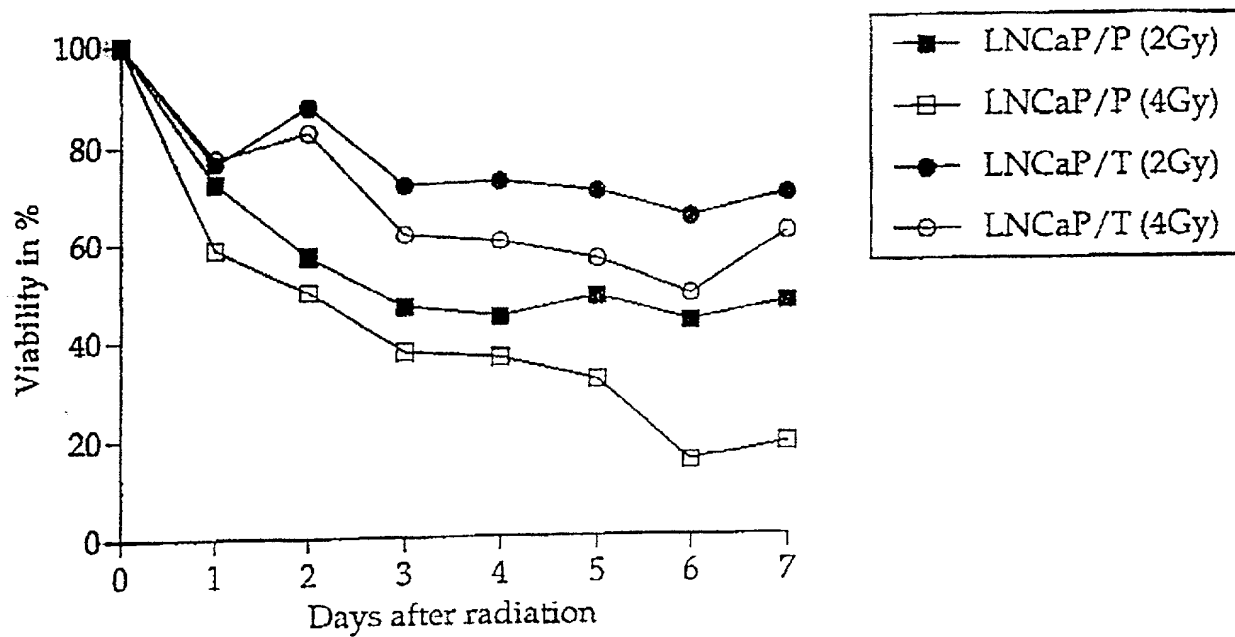


Fig 9B

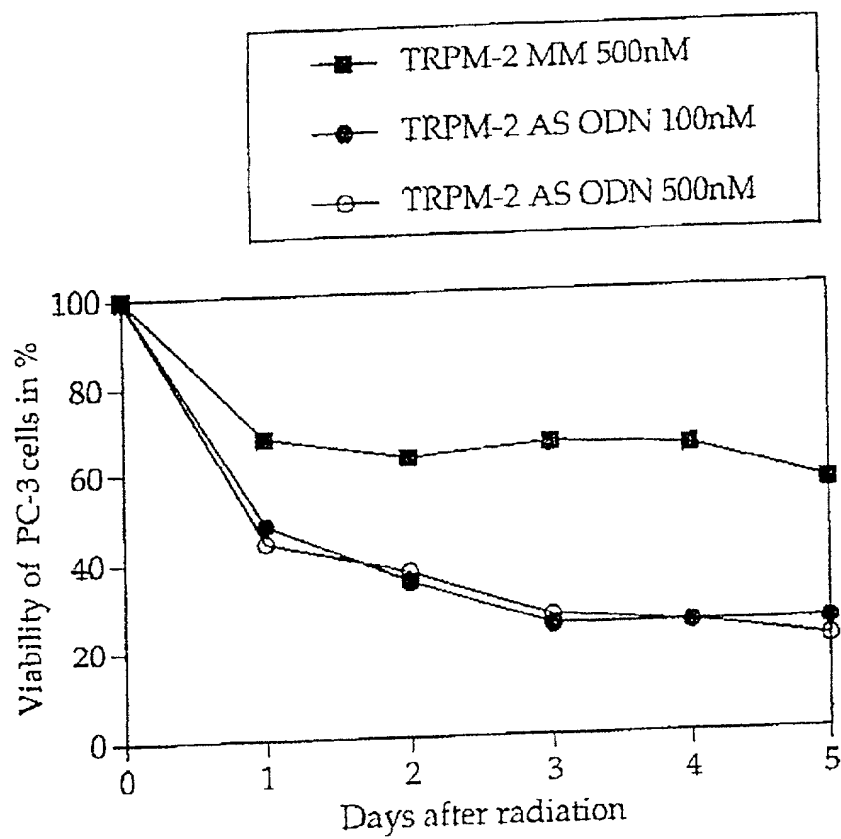


Fig. 10

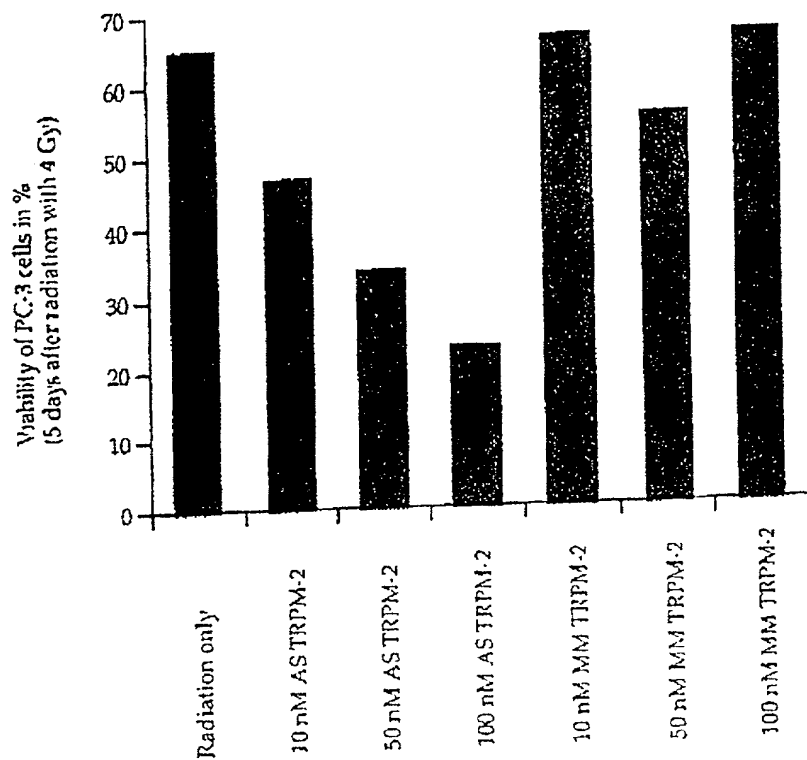


Fig 11A

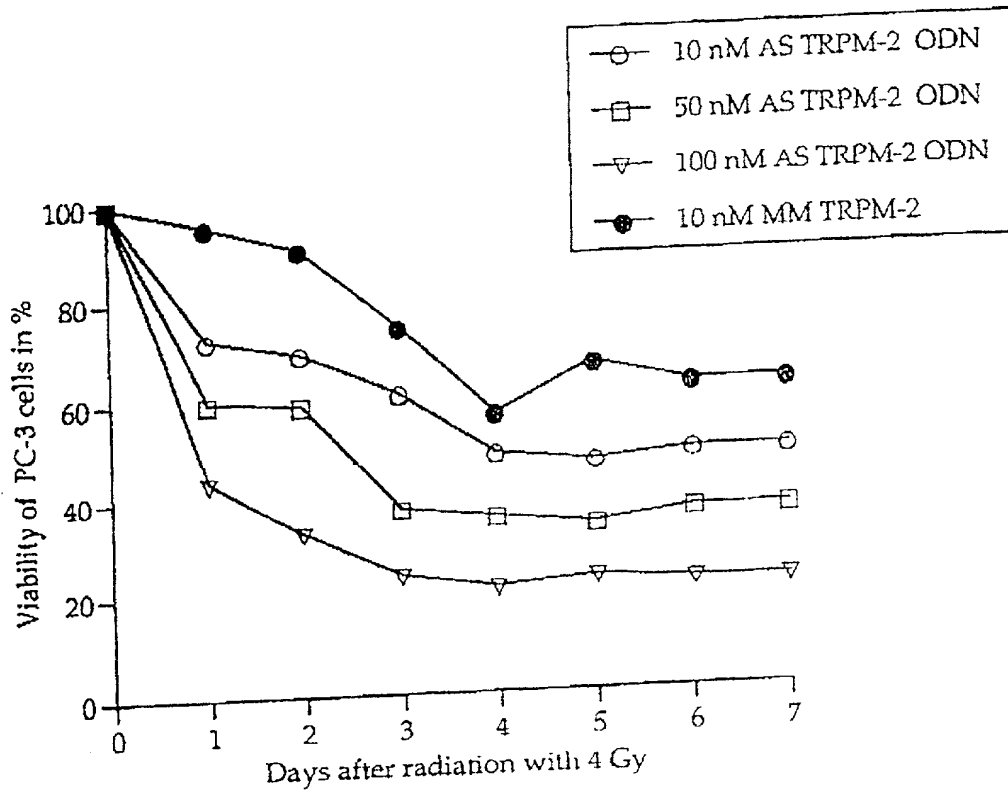


Fig. 11B

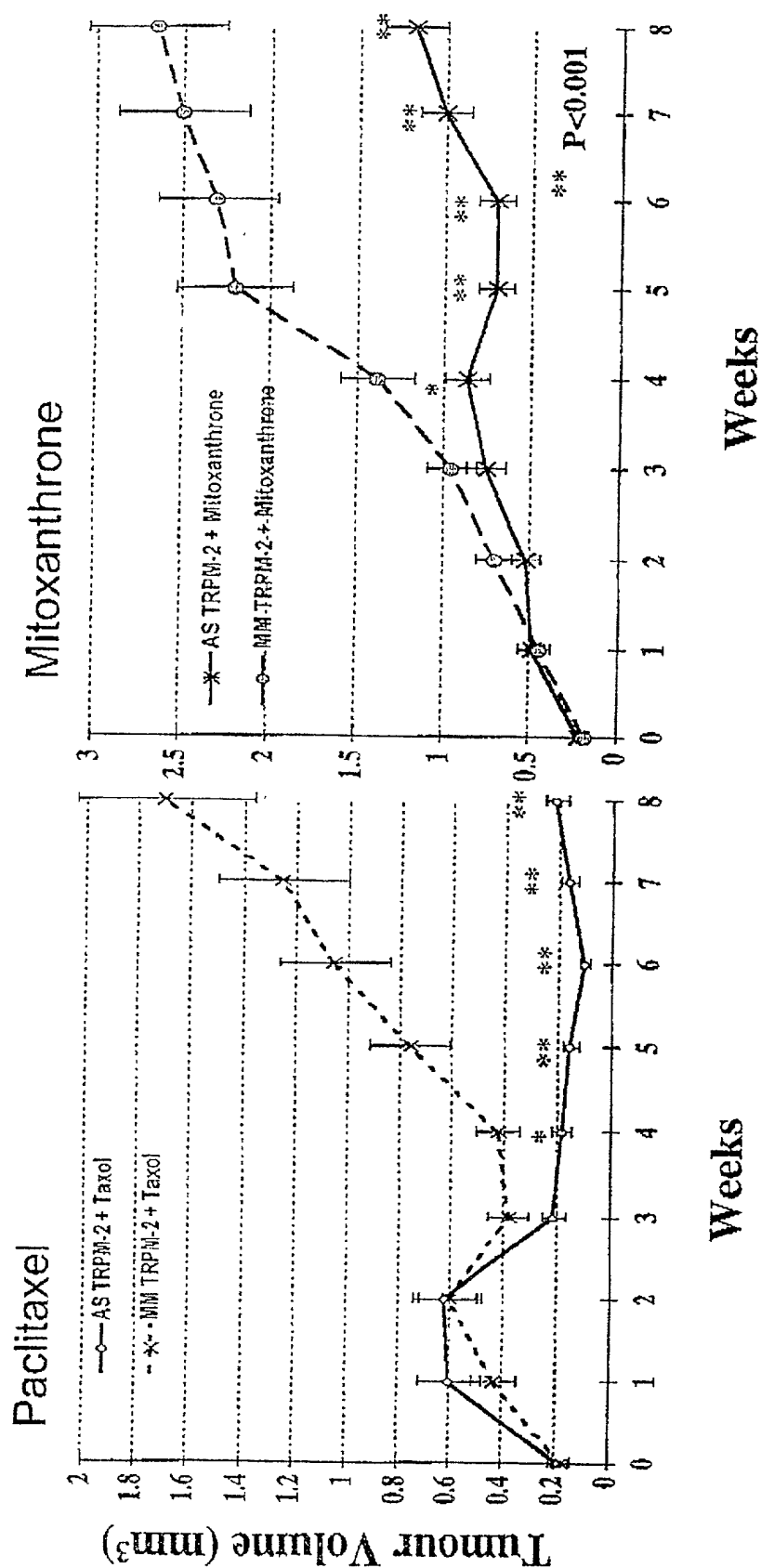


Fig. 12B

Fig. 12A

**COMBINED DECLARATION  
AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that:

My citizenship, residence and post office address are as listed below next to my name.

I believe I am the original, first and ☐ sole/☒ joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: **TRPM-2 Antisense Therapy**

the specification of which

(a) ☐ is attached hereto.

(b) ☐ was filed on \_\_\_\_\_ as Application Serial No. \_\_\_\_\_ and was amended on \_\_\_\_\_.

(c) ☒ was described and claimed in International Application No. PCT/US00/04875 filed on February 25, 2000 and amended on March 20, 2001.

**Acknowledgment of Duty of Disclosure**

I hereby state that I have reviewed and understood the content of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56(a).

**35 U.S.C. § 120**

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status)(patented,pending,abandoned)	(Patent No. if applicable)
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(Application Serial No.)	(Filing Date)	(Status)(patented,pending,abandoned)	(Patent No. if applicable)
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**Power of Attorney**

I hereby appoint Carl Oppedahl, PTO Reg. NO. 32,746, Marina T. Larson, PTO Reg. No. 32,038, and D'Arcy Straub, PTO Reg. No. 47,113, of the firm of OPPEDAHL & LARSON LLP, having office at P.O. Box 5068, Dillon, CO 80435-5068 as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO:



**021121**

PATENT TRADEMARK OFFICE

DIRECT TELEPHONE CALLS TO:

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(970)468-6600

### Claim for Priority

I hereby claim foreign priority benefits under 35 U.S.C. § 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign applications for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

EARLIEST FOREIGN APPLICATION(S), FILED WITHIN TWELVE MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION					
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)	PRIORITY CLAIMED	CERTIFIED COPY ATTACHED
				YES[ ] NO[ ]	YES[ ] NO[ ]
FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION					
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)		

### Provisional Application

I hereby claim the benefit under 35 U.S.C § 119(e) of any United States provisional application(s) listed below.

60/121,726

February 26, 1999

(application number)

(filing date)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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DATE June 7/01		SIGNATURE	

- [X] Signature for additional joint inventor attached. Number of Pages 1.
- [ ] Signature by Administrator(trix) or legal representative for deceased or incapacitated inventor. Number of Pages     .
- [ ] Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR § 1.47. Number of Pages     .

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DATE June 8/01		SIGNATURE Paul Rennie	
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POST OFFICE ADDRESS		CITY	STATE/COUNTRY ZIP CODE
DATE		SIGNATURE	

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Nelson, Colleen

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